Rapid *in vitro* cross-resistance evolution to different phages of the phytopathogenic bacterium *Rastonia pseudosolanacearum*

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- Biological model: *Ralstonia solanacearum* species complex
- Bacteria type: soil-borne gram-negative
- Disease: bacterial wilt
- Host range: over 200 crops and wild plant species
- Distribution: worldwide especially in tropical regions

How long does it take *Ralstonia pseudosolanacearum* to acquire phage resistance?

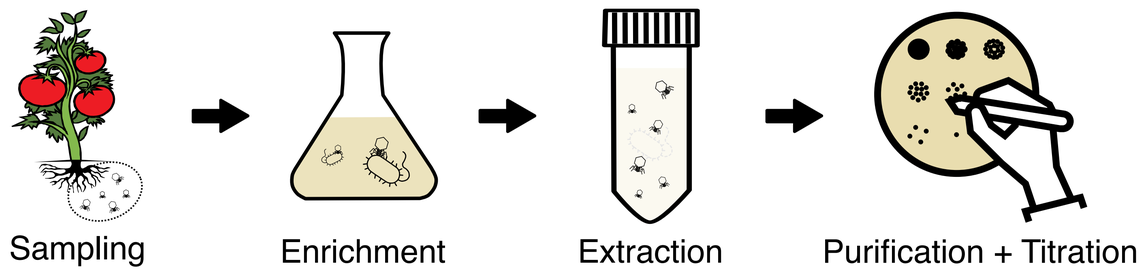
1 Materials

Ralstonia pseudosolanacearum
Origin: Reunion island
Phylotype-sequevar: I-31
Haplotype: MT035
Strain: Run3012

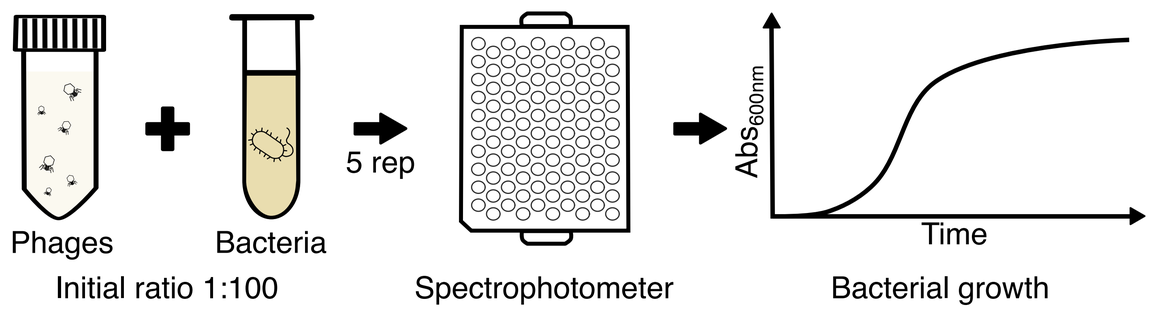
Phages population
Origin: Reunion island
Sampling: root, soil, irrigation water
Number of phages: 10
Classification: 4 species, 3 genera, 2 families

2 Methods

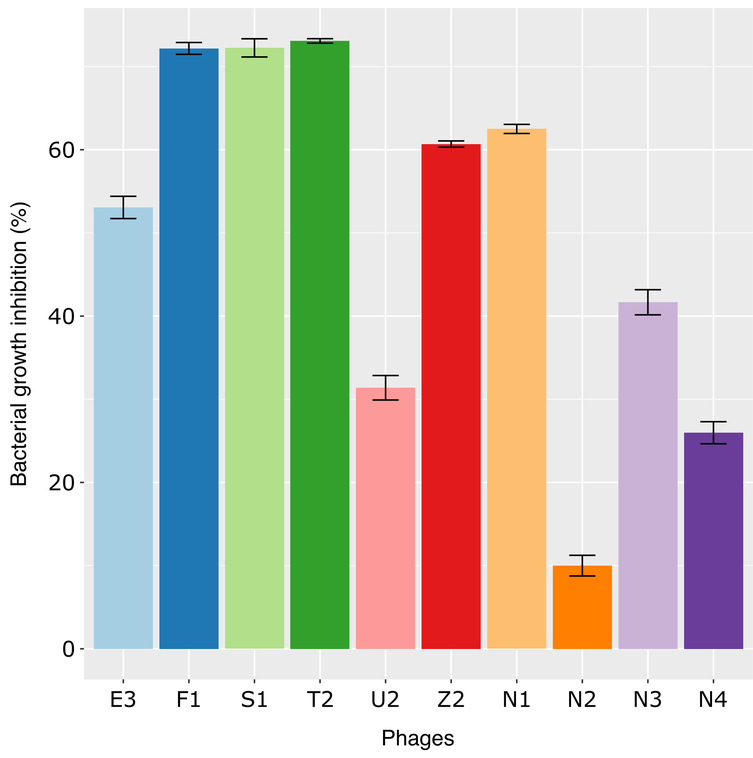
Phages isolation



Measurement of bacterial growth dynamics



3 Virulence



Bacterial growth inhibition over 24h

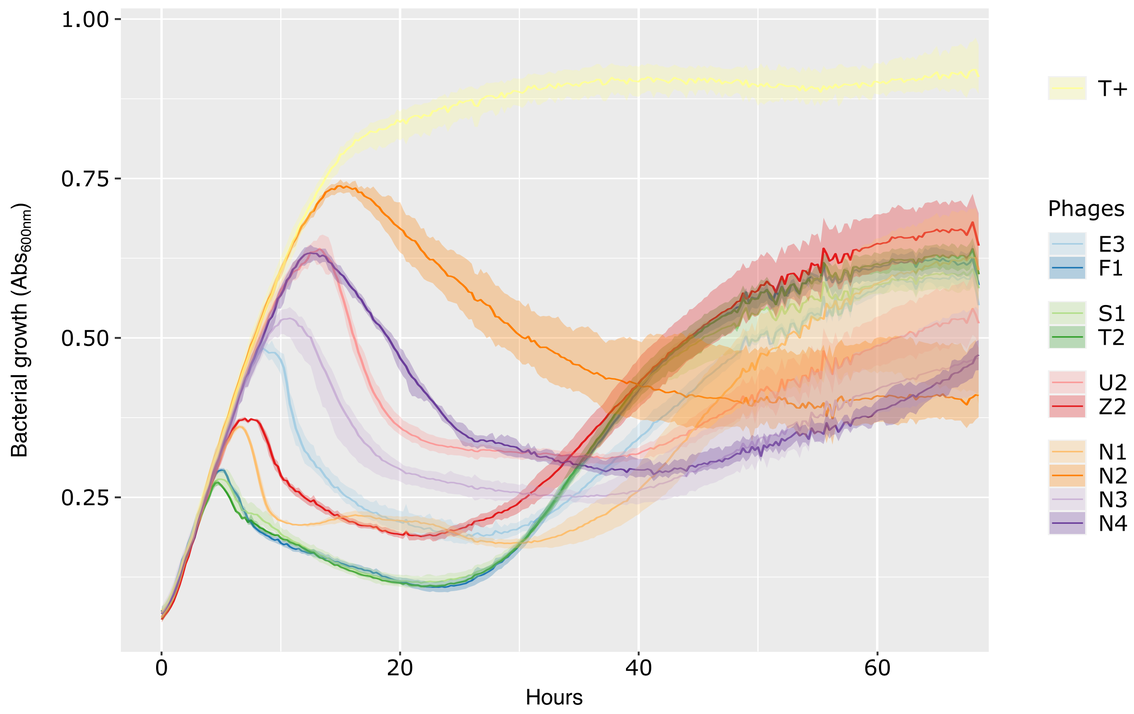
7/10 phages reduce bacterial growth (>50%)

Variability of virulence according to phages

No link with phages species

Good *in vitro* efficiency against strain Run3012

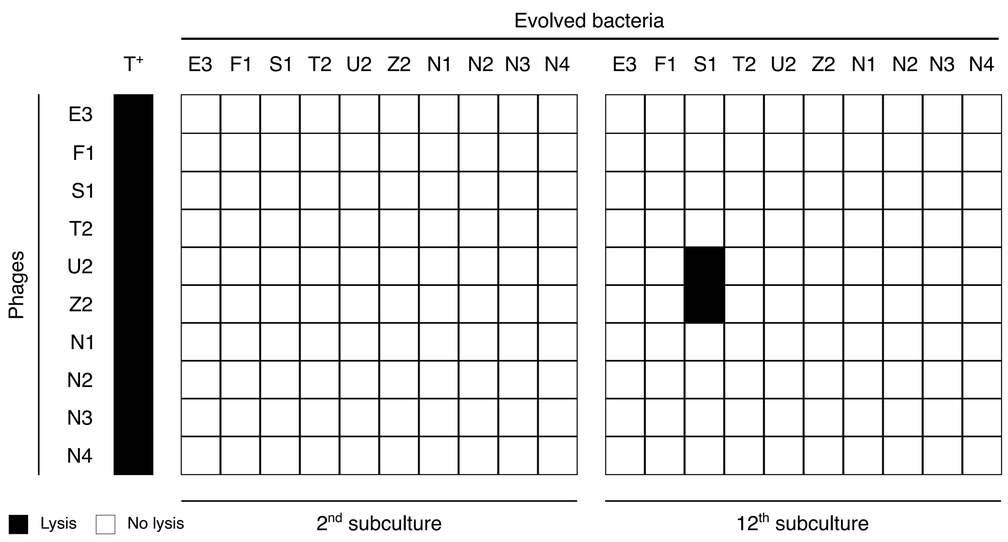
4 Bacterial growth dynamics



Bacterial growth usually restarts after 24 hours

Possible emergence of bacterial resistance

5 Cross-resistance

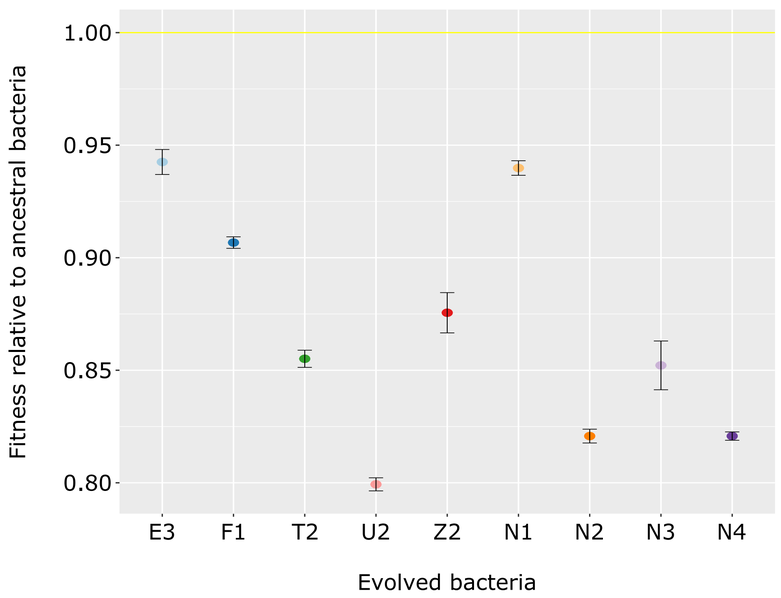


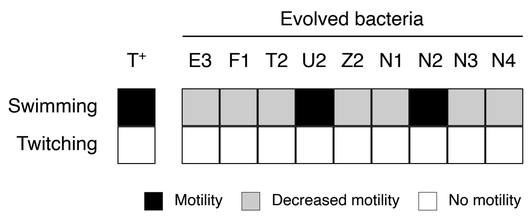
Absence of clear lysis plaques

Resistance against all phages (encountered and others)

9/10 evolved bacteria maintain their resistance after 12 subcultures

6 Fitness tradeoffs





Reduction in growth rate compared to ancestral bacteria

No link with the encountered phages species

Absence of twitching motility in the ancestral and evolved bacteria (pili-dependent movement)

Presence of swimming in the ancestral bacteria (flagellum-dependent movement)

Swimming ability loss for 8/10 evolved bacteria

Perspectives

DNA sequencing to elucidate the genetic basis of bacterial resistance

Resistance cost associated with reduced bacterial growth and motility

Testing phages with different caracteristics or complementary strategies for bacteria biocontrol

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